IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of:

Frank Portugal — Examiner: J. Souaya

Serial No.: 09/027,439 — Group Art Unit: 1655

Filed: February 20, 1998

Title: COMPOSITIONS AND METHODS FOR DIFFERENTIATING AMONG SHIGELLA SPECIES AND SHIGELLA FROM E. COLI SPECIES

RESPONSE UNDER 37 CFR §1.111

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

The application above was unintentionally abandoned for failure to respond to the Office Action dated September 13, 2001. The entire delay in responding was unintentional. Attached is a petition to revive the application and the petition fee. The following is a reply to the Office Action.

Please amend the above identified application as indicated below and consider the remarks which follow.

IN THE CLAIMS

Please amend claims 37-39 as follows.

37. (Amended) A nucleic acid molecule consisting essentially of 10 to 40 sequential nucleotides from SEQ ID NO:7 which include an inserted nucleotide U or T in between positions 88 and 89 of either 16s ribosomal RNA or 16 s ribosomal DNA, said positions being with reference to an E. coli equivalent position of SEQ ID NO:7,

or an RNA equivalent thereof,

E1

or a nucleic acid molecule complementary to said molecule.

38. (Amended) A nucleic acid molecule consisting essentially of 10 to 40 sequential nucleotides selected from the group consisting of sequences of nucleotides in Table 2 which include (a) nucleotide C at position 964 or (b) a deletion at position 978 of either 16s ribosomal RNA or 16 s ribosomal DNA, said positions being with reference to an *E. coli* equivalent position of SEQ ID NO: 7,

E1

or an RNA equivalent thereof,

or a nucleic acid molecule complementary to said molecule.

39. (Amended) A nucleic acid molecule consisting essentially of 10 to 40 sequential nucleotides selected from the group consisting of sequences of nucleotides in Table 2 which include nucleotide A at position 76 of either 16S ribosomal RNA or 16S ribosomal DNA, said position being with reference to an *E. coli* equivalent position of SEQ ID NO: 7,

or an RNA equivalent thereof,

or a nucleic acid molecule complementary to said molecule.

Please add new claims 45-56 as follows:

--45. A nucleic acid molecule consisting essentially of 10 to 40 sequential nucleotides selected from the group consisting of sequences of nucleotides in Table 2 which include (a) nucleotide C at position 1006, or (b) nucleotide G at position 1009 or (c) nucleotide T at position 1110 or (d) nucleotide A at position 1017 or (e) an inserted nucleotide U or T in between positions 1020 and 1021, or (f) nucleotide G at position 1022 of 16s ribosomal RNA or 16s

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ribosomal DNA, respectively, said positions being with reference to an *E. coli* equivalent position of SEQ ID NO: 7,

or an RNA equivalent thereof,

or a nucleic acid molecule complementary to said molecule.

46. A nucleic acid molecule consisting essentially of 10 to 40 sequential nucleotides selected from the group consisting of sequences of nucleotides in Table 2 which include nucleotide C at position 92 of 16s ribosomal RNA or 16s ribosomal DNA, respectively, said position being with reference to an *E. coli* equivalent position of SEQ ID NO: 7,

or an RNA equivalent thereof,

or a nucleic acid molecule complementary to said molecule.

47. An isolated nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6,

or an RNA equivalent thereof,

or a nucleic acid complementary to said isolated molecule.

48. An isolated nucleic acid molecule consisting essentially of a nucleotide sequence as set forth in SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 5, or SEQ ID NO: 6,

or an RNA equivalent thereof,

or a nucleic acid complementary to said isolated molecule.

49. A nucleic acid molecule comprising a nucleotide sequence as set forth in SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16. or SEQ ID NO: 17,

or an RNA equivalent thereof,

or a nucleic acid complementary to said molecule.

50. A nucleic acid molecule consisting essentially of sequential nucleotides selected

from the group consisting of SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 14, SEQ ID NO: 16. and SEQ ID NO: 17,

or an RNA equivalent thereof,

or a nucleic acid complementary to said isolated molecule.

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51. A kit comprising 2 or more nucleic acid molecules of claim 50.

52. An isolated nucleic acid molecule comprising a nucleotide sequence of SEQ ID NO: 6, or a nucleic acid molecule complementary to said isolated molecule or an RNA equivalent thereof.

REMARKS

The amendments to claims 37 through 39 serve to define the structure of the molecules more particularly by using more conventional language such as "consisting essentially of" and "selected from the group consisting of."

The molecules defined in claims 37-39 and 45-46 do not exist in nature and therefore, there is no need to restrict these claims to isolated or purified molecules.

The functional limitations recited in original claims such as identifying the Shigella *sonnei*, *Shigella dysenteriae* or *Shigella boydii* species and distinguishing the "E. coli and other Shigella species " are not needed to define the molecules of claims 37-39 and 45-46.

New claim 45 conforms to original claim 40 but recites the identifying nucleotides found in Table 2 rather than referring to "an identifying nucleotide in region 1001-1041."

New claim 46 conforms to previously canceled claim 41.

New claims 47-50 conform to previously canceled claims 42 and 43 but contain the conventional language introduced to pending claims 37-39.

New claims 49 and 50 are comparable to previously canceled claim 43. New claims 49 and 50 do not recite SEQ ID NOS. 18, 19 and 20. The sequences of SEQ ID NOS. 18, 19 and 20 are identical to the sequences of SEQ ID NOS. 1, 2 and 3 that are claimed in related application Serial number 09/027,089.

New claim 51 is a kit claim comprising one or more of the nucleic acid molecules of claim 50. Support for this claim is found on page 19, lines 20-23.

New claim 52 is directed to molecule comprising the nucleotide sequence of SEQ ID NO: 6 or a nucleic acid molecule complementary thereto or an RNA equivalent thereof. Support for this claim is found on page 40 of the specification.

Obviousness rejection

Claims 37-39 were rejected in the Office Action dated September 13, 2001 in view of Cilia et al or Hogan et al (U.S. Patent 5,714,321) in combination with Faruque et al. (J. Clinical Microbiology, 1992, vol. 30, pp. 2996-2999).

In the Office Action dated December 20, 2000, cancelled claims 40-42, which correspond to new claims 45-48 herein, were also found to be obvious in view of the Cilia et al or Hogan et al in combination with Faruque et al., the same references relied on in rejecting claims 37-39.

Claim 43, which conforms to new claims 49 and 50, was rejected in the Office Action dated December 20, 2000 as allegedly anticipated by Cilia et al.

These rejections are traversed.

No functioning probes or primers have been identified or relied on in rejecting the nucleic acid molecules claimed herein as obvious. Furthermore, no probes or primers in the prior art have been shown to have a structure similar to the nucleic acid molecules defined in claims 37-39 and 45-50. Instead, the obviousness rejections are based on the allegation that it would be obvious to one of ordinary skill in the art to construct the claimed molecules from the known 16s rRNA and 16s rDNA sequences of the Shigella species and E. coli since such methods allegedly were readily known in the art. It is also alleged that one skilled in the art would be motivated to prepare the claimed nucleic acid molecules to identify and differentiate E. coli from Shigella.

Applicants claim new nucleic acid molecules defined by a unique structure. A prima facie case of unpatentability for these claims based on obviousness requires the teachings of the prior art provide a suggestion or motivation for a person of ordinary skill to make the modifications necessary to form the claimed compounds, In re Laskowski, 871 F. 2d 115, 10 USPQ 2d (Fed. Cir. 1989), In re Mills, 916 F.2d 680, 16 USPQ 2d 1430 (Fed. Cir. 1990). The prior art relied on provides neither. Hogan et al. describes probes for detecting salmonella having a structure completely unrelated to the molecules claimed herein. Cilia discloses large portions of the rRNA sequences for Shigella flexneri, Shigella dysenteriae, Shigella sonnei and E. coli but provides no suggestion or direction to prepare small molecules of 10-40 nucleotides having the specific nucleotides and sequences defined in the claims herein. Molecules consistent with SEQ. ID. NO. 6 are not shown.

The published sequences referred to in Applicants' specification add nothing to the teachings of Hogan et al and Cilia to suggest or lead one skilled in the art to the claimed molecules. Although some of the molecules claimed herein were derived from portions of

known rDNA sequences for the E. coli and Shigella species, there is no suggestion or direction from the teachings of the cited references (Cilia, Hogan and Faruque) to select the portions of these known rDNA sequences from which these nucleic acid molecules were derived.

Whether the procedure for obtaining the claimed molecules is "readily known," as alleged in the Office Action, is not relevant to the patentability determination for these claimed molecules. The statute which defines the "nonobvious" condition for patentability, 35 USC §103, expressly excludes the consideration of such methods in stating, "Patentability shall not be negatived by the manner in which the invention was made." This principle was followed in In re Kratz and Strasburger, 201 USPQ 71 (1979 CCPA) and In re Deuel 34 USPQ2d 121 (Fed. Cir. 1995).

In Kratz, claims to an isolated naturally occurring compound found in strawberries were found to be patentable although the abstract process of discovering flavor components, i.e., chemically searching the strawberry, was found to be "unquestionably obvious."

The Applicants in Deuel claimed specific molecules (DNA and cDNA), which were rejected in view of known methods for obtaining them (gene cloning). In reversing the rejection, the Federal Circuit stated in various portions of the decision:

> The PTO's focus on known methods for potentially isolating the claimed DNA molecules is also misplaced because the claims at issue define compounds, not methods." (at page 1215);
>
> ...the existence of a general method of isolating cDNA or DNA molecules is essentially irrelevant to the question whether the specific molecules themselves would have been obvious, in the absence of other prior art that suggests the claimed DNAs. (at page 1215); and
>
> The fact that one can conceive a general process in advance for preparing an *undefined* compound does not mean that a claimed specific compound was precisely envisioned and therefore obvious. (sat page 1216).

Even if, as the Examiner alleges, "it would be obvious for the ordinary artisan to construct probes and primers to regions of variability" and these methods were "readily known," this is not enough information to render the claimed nucleic acid molecules obvious. These general methods for preparing undefined probes and primers do not make the specific molecules claimed herein obvious. A number of "regions of variability" can be found in comparing the reference E. coli strain and other Shigella strains, such as those disclosed by Cilia. Nothing in these "readily known" methods has been shown to lead one skilled in the art to the claimed molecules. Using embodiments in Claim 38 as an example, there is no motivation or suggestion to prepare molecules having i) 10-40 nucleotides, ii) nucleotide C at position 964 and iii) a sequence of nucleotides consistent with the sequences in Table 2 which have nucleotide C at position 964. Nothing in the prior art of record would have motivated an artisan of ordinary skill to select, among the myriad of possible "regions of variability" within the known rDNA sequences, the particular regions from which Applicants prepared the claimed nucleic acid molecules of 10-40 nucleotides with specific sequences that include specific distinguishing nucleotides.

As to the alleged motivation to construct the molecules of the claimed invention so as to obtain probes and primers that identify and differentiate E. coli from Shigella, this is no more than a general incentive to try to prepare such probes and primers. There is no evidence of motivation to prepare the particular molecules claimed herein. As stated in In re Deuel, supra,

> "Obvious to try" has long been held not to constitute obviousness. In re O'Farrell, 7 USPQ2D 1673, 1680-81 (Fed. Cir. 1988). A general incentive does not make obvious a particular result, nor does the existence of techniques by which those efforts can be carried out.

Furthermore, there is evidence within the Cilia reference that identifying regions of variability by conventional methods would be not enough to provide probes which distinguish E. coli from Shigella species. Cilia disclose at page 459, col. 1, lines 50-53 and extending to col. 2, lines 1-7,

> Finally, one should note that some care should be taken when deriving species-specific probes, especially for detection by PCR, as a single operon

> in one species might bear a sequence that is present in the majority of the operons in another species (see Fig. 3). How often such problems can be encountered is still difficult to assess, because we lack data for individual operon sequences in closely related organisms and because we only have poor estimates for the frequencies of recombinations for rrn operons.

It is also noted that despite analyzing sequence variations among E. coli and Shigella species, Cilia et al. did not obtain functioning probes. Cilia disclose at page 456, right column, last paragraph:

> However, further analyses could not derive decisive polyphylogenetic ingroup relationships within the (*Escherichia* + *Shigella*) and *Salmonella* clades, at least on the basis of SSU rRNA sequences analysis.

The prior art, when considered as a whole, teaches that identification of the regions of variability in the sequences for E. coli and Shigella species is not enough to obtain functional probes. As noted above, even if such identification alone were sufficient, the claims still would not be obvious. Where as here, such sufficiency is lacking, it is clearer that for there to be the necessary motivation to prepare the claimed molecules to differentiate E. coli from Shegella, there must be particular direction in the prior art which suggests these specific molecules. No such evidence or suggestion in the art has been provided.

In that the prior art provides no hint or suggestion of the structure of the claimed molecules and also provides no motivation to prepare these molecules, Applicants maintain they are unobvious and that all rejections under 35 U.S.C.§ 103 should be withdrawn.

Claims 37-39, 45 and 46

Some of these claims define molecules with a sequence of nucleotides not present in any rDNA sequence of record, e.g. claim 38. The sequence of this molecule could not have been predicted based on the sequences known in the art. Therefore, the subject matter of claim 38 is further distinguished from the prior art in that the sequence of nucleotides itself is unknown and unobvious.

Some of the nucleic acid molecules defined by these claims were derived from known

rDNA sequences. Although the source rDNA sequence may have been known, these molecules are unobvious since it would not have been obvious to compare and/or select the regions of rDNA from which these molecules are derived. For example, Hogan et al. (U.S. Patent 5,714,321) states at Col 5, lines 54-61:

> Generally, only a few regions are useful for distinguishing between closely related species of a phylogenetically conserved genus, for example, the region of 400-500 bases from the 5' end of the 16S rRNA molecule. An analysis of closely related organisms (Figs. 6, 7 and 8) reveals the specific positions (variable regions) which vary between closely related organisms. These variable regions of rRNA molecules are likely candidates for probe design.

and at Col 6, lines 11-15:

> These figures also shown [sic] that the differences are distributed across the entire 16S and 23S rRNA's. Many of the differences, nonetheless, cluster into a few regions. These locations in the rRNA are good candidates for probe design, with our current assay conditions.

In addition, in the paragraph bridging columns 1 and 2 of page 457, Cilia states:

> Most of the variable positions that have been observed from one operon to another are located in domains of high mutation rates. These highly divergent domains are usually excluded from the data matrix when a phylogenetic analysis is undertaken for resolving distant relationships because these positions would include too many characters that are obviously homoplasic. It is therefore not important that the existence of a heterogeneity is not known or is not taken into account, provided that these characters are removed from phylogenetic analyses.

In the language cited above, Hogan teaches that in forming probes for distinguishing closely related species, only a few regions of the rRNA are useful and Cilia teaches that certain highly divergent domains may be ignored in a phylogenetic analysis. There is no direction in the prior art as to which regions of the known rDNA sequences for the Shegella/E. coli species would provide effective probes and there is clearly no direction to select the regions of these known rDNA sequences from which some of the claimed molecules are derived. The comparable regions (using E. coli as a reference) within the rRNA sequences investigated by

Cilia were either ignored or the differences in the nucleotides within these regions were undetected or unreported. For example, Cilia do not make comparisons in the region of 961-991, where the identifying nucleotides for the molecules defined in claim 38 are located. Similarly, Cilia do not compare the sequences of E. coli and *Shigella boydii* and identify the distinguishing nucleotide at position 92, which defines the molecules within claim 46 or the distinguishing nucleotide at position 76, which defines the molecules within claim 39. Although Cilia compares sequences in the region of 1003 to 1033 and 3-95, the distinct nucleotides recited in claims 37 and 45 are not mentioned. Cilia report no differences between E. coli and the *Shigella sonnei*, *Shigella dysenteriae* or *Shigella flexneri* species at positions 88 and 89, which define molecules of claim 37. Similarly, Cilia fails to identify distinguishing nucleotides at positions 1006, 1009, 1010, 1017, 1022, and the nucleotides between positions 1020 and 1021, which define molecules within claim 45.

Therefore, when considering the prior art as a whole, one of ordinary skill in the art would not be motivated to prepare the nucleic acid molecules of claims 37, 39 and 45-50 derived from known sequences in that there is no direction or suggestion to select the region of these known sequences from which these molecules are derived and would clearly not be motivated to prepare the nucleic acid molecules of claim 38, derived from a sequence not mentioned in the cited references.

The Anticipation rejection

The Office Action dated December 20, 2000 alleges that claims 43 and 44 (now claims 47 -50) are anticipated by Cilia. None of the sequences disclosed in Cilia (or in any prior art of record) are identical to the sequence of the instantly recited nucleic acid molecules. Rather, the instant inventors were the first to clone and sequence the individual 16S rDNA's recited in claims 47 and 48. SEQ ID NOS. 3, 4, 5 and 6 recited in those claims are from the cloned 16s rDNAs from Shigella flexneri, sonnei, dysenteriae and boydii, respectively. Claims 49 and 51, which recite fragments of these sequences, are also novel. Therefore, there is no basis for the anticipation rejection.

Not only are the sequences recited in claims 47 and 48 not anticipated, but they are also non-obvious over any sequences in the prior art of record. No evidence has been provided that the sequences of any of these nucleic acids were suggested in the prior art at the time the invention was made or that the prior art provides direction to obtain these specific sequences. The sequences of Shigella rRNAs presented in Cilia, for example, are composite sequences. By contrast, the Shigella SEQ IDs of the instant invention reflect individual clones of individual 16s rRNA genes. The specific sequences of the instantly claimed nucleic acids cannot be contemplated or conceived based on, *e.g.*, such composite sequences. "What cannot be contemplated or conceived cannot be obvious." *In re Deuel, supra.* See also *In re Bell*, 26 USPQ2d 1629 (Fed. Cir. 1993).

In view of the above remarks, withdrawal of the rejections and allowance of claims 37-39 and 45-56 are earnestly solicited. The Commissioner is hereby authorized to charge any fees associated with this response or credit any overpayment to Deposit Account No. 13-3402.

Respectfully submitted,

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Attorney Docket No.: CABTECH 2

Date: June 12, 2002

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